

Map of pRM1

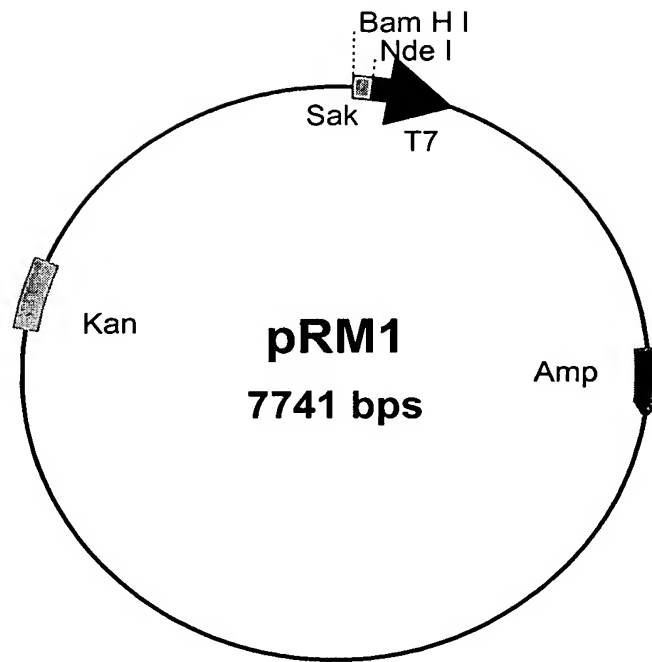


Fig. 1

Fig. 2 Sequence of oligonucleotide to construct the protein expression cassette

1. 5' GATCAAGCTTATCATCGATAAGCTTACAGGACGCTGGGTAAAA
GTATTT -3" (51 mer) PEC 1
2. 5"-
5 ATCTTATTGACCTCTCAAACTTAATCCACATCAAACTCAAATAC
TTTAAACCC -3" (55 mer) PEC 2
3. 5"-AGAGGTCAATAAGATTATAATATGTGATGCTTCACAATTCTGATG
TATGGCAAAA -3" (55 mer) PEC 3
4. 5"-ATGAGGTCTTCCTTAAGTTCATTATTATGGTTTTGCCATACATCA
10 GAATT -3" (50 mer) PEC 4

Fig. 3 Complete nucleotide sequence of expression cassette, OXY-1

GATCAAGCTTATCATCGATAAGCTTACAGGACGCTGGGTAAAAAGTATTTG
AGTTTTGATGTGGATTAAGTTTTGAGAGGTCAATAAGATTATAATATGTGA
15 TGCTTCACAATTCTGATGTATGGCAAAACCATAATAATGAACTTAAGGAAG
ACCTCATG (161 mer)

Fig.4 Nucleotide sequence of SAK gene

20 TCAAGTTCATTCGACAAAGGAAAATATAAAAAGGGCGATGACGCGAGTTA
TTTTGAACCAACAGGCCCGTATTTGATGGTAAATGTGACTGGAGTTGATGG
TAAAGGAAATGAATTGCTATCCCCTCATTATGTCGAGTTTCCTATTAAACC
TGGGACTACACTTACAAAAGAAAAAATTGAATACTATGTCGAATGGGCAT
TAGATGCGACAGCATATAAAGAGTTTAGAGTAGTTGAATTAGATCCAAGC
25 GCAAAGATCGAAGTCACTTATTATGATAAGAATAAGAAAAAAGAAGAAAC
GAAGTCTTTCCCTATAACAGAAAAAGGTTTTGTTGTCCCAGATTTATCAGA
GCATATTAAAAACCCTGGATTCAACTTAATTACAAAGGTTGTTATAGAAAA
GAAATAA
(411 nucleotides)

30

Fig.5 Nucleotide sequence of SAK 1 gene

GAACTTAAGGAAGATATACATATGTCAAGTTCATTCGACAAAGGAAAATA
 TAAAAAGGGCGATGACGCGAGTTATTTTGAACCAACAGGCCCGTATTTGAT
 GGTAAATGTGACTGGAGTTGATGGTAAAGGAAATGAATTGCTATCCCCTCA
 5 TTATGTCGAGTTTCCTATTAAACCTGGGACTACACTTACAAAAGAAAAAAT
 TGAATACTATGTCTGAATGGGCATTAGATGCGACAGCATATAAAGAGTTTA
 GAGTAGTTGAATTAGATCCAAGCGCAAAGATCGAAGTCACTTATTATGATA
 AGAATAAGAAAAAAGAAGAAACGAAGTCTTTCCTATAACAGAAAAAAGGT
 TTTGTTGTCCCAGATTTATCAGAGCATATTAAAAACCCTGGATTCAACTTA
 10 ATTACAAAGGTTGTTATAGAAAAGAAATAAAACAAAATAGTTGTTTATTAT
 AGAAAGTAATGTCTTGATTGAATATGTGTAGTGAAATTATCTTTCATCAAA
 TTCTCATTCATGCACGAATGGTTCTGCCCCACCTAATCAGATATTACGTGA
 CTTATGGGGAGAAATCAGTTTGGATAAAAGTGGAGGATCCAGTAGCCG (

606 nucleotides)

15 Oligo's :

SAK-3 primer :

5'- GAACTTAAGGAAGATATACATATGTCAAGTTCATTCGACAAAGGA-3'

(45 mer)

SAK-2 primer :

20 5'- CGGCTACTGGATCCTCCACTTTTATCCAAACTGATTT -3' (38 mer)

Fig.6 Nucleotide sequence of SAK 2 gene

GAACTTAAGCATATGAAAGGAAAATATAAAAAGGGCGATGACGCGAGTTA
 TTTTGAACCAACAGGCCCGTATTTGATGGTAAATGTGACTGGAGTTGATGG
 TAAAGGAAATGAATTGCTATCCCCTCATTATGTCTGAGTTTCCTATTAAACC
 5 TGGGACTACACTTACAAAAGAAAAAATTGAATACTATGTCTGAATGGGCAT
 TAGATGCGACAGCATATAAAGAGTTTAGAGTAGTTGAATTAGATCCAAGC
 GCAAAGATCGAAGTCACTTATTATGATAAGAATAAGAAAAAGAAGAAAC
 GAAGTCTTTCCCTATAACAGAAAAAGGTTTTGTTGTCCCAGATTTATCAGA
 GCATATTAAAAACCCTGGATTCAACTTAATTACAAAGGTTGTTATAGAAAA
 10 GAAATAAAACAAAATAGTTGTTTATTATAGAAAGTAATGTCTTGATTGAAT
 ATGTGTAGTGAAATTATCTTTCATCAAATTCTCATTCATGCACGAATGGTTC
 TGCCCCACCTAATCAGATATTACGTGACTTATGGGGAGAAATCAGTTTGGA
 TAAAAGTGGAGGATCCAGTAGCCG (581 nucleotides)

Oligo's :

15 SAK-4 primer :

5'- GAACTTAAGCATATGGCTGGAGCTTATAAAAAGGGC-3' (36 mer)

SAK-2 primer:

2. 5'- CGGCTACTGGATCCTCCACTTTTATCCAAACTGATTT -3' (38 mer)

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25

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Fig. 7 Schematic representation of construction of plasmid , pOXYPRO, carrying OXY-1 expression cassette.

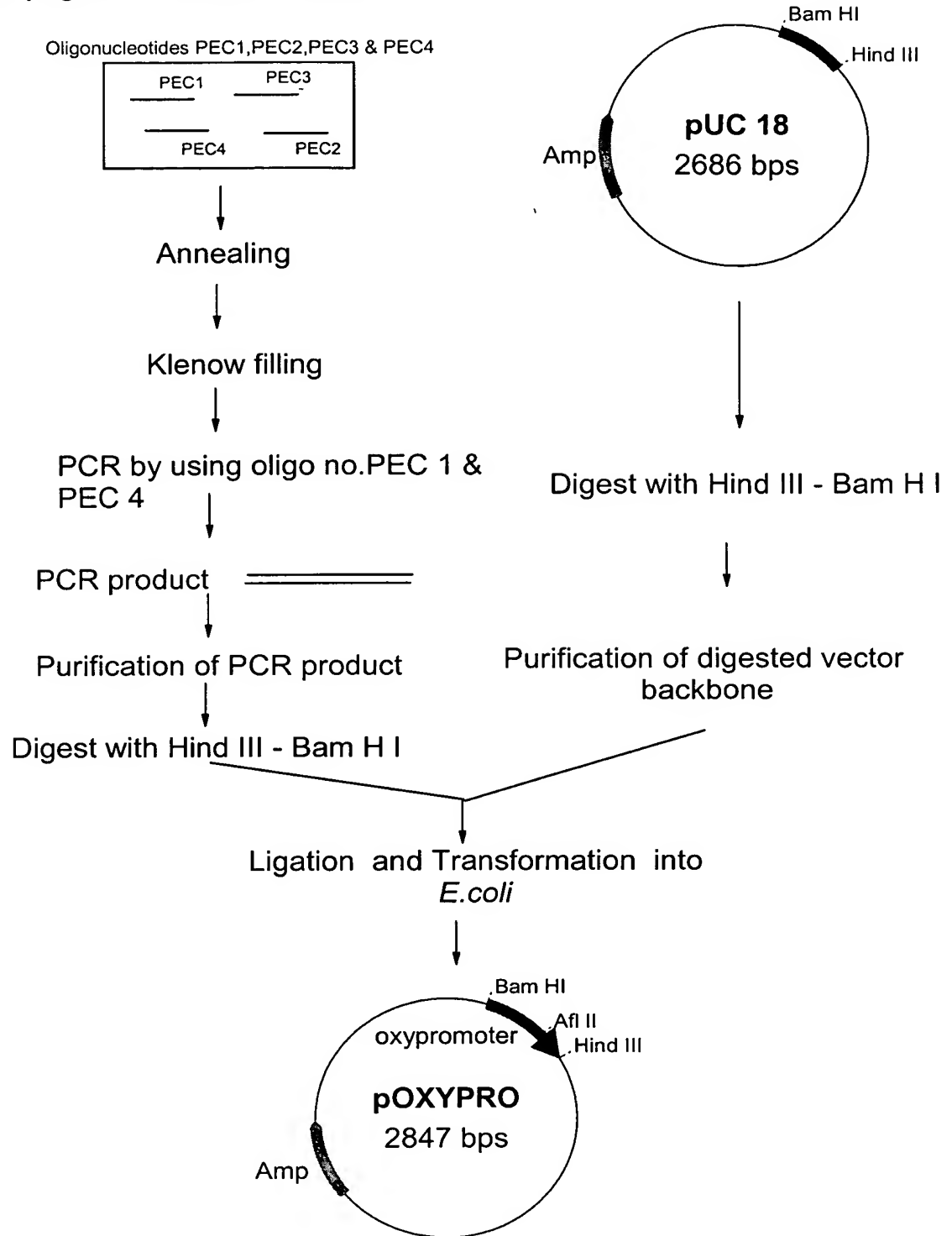


Fig. 8 Construction and map of plasmid, pOXYSAK-1 containing SAK-1 gene under OXY-1 expression cassette.

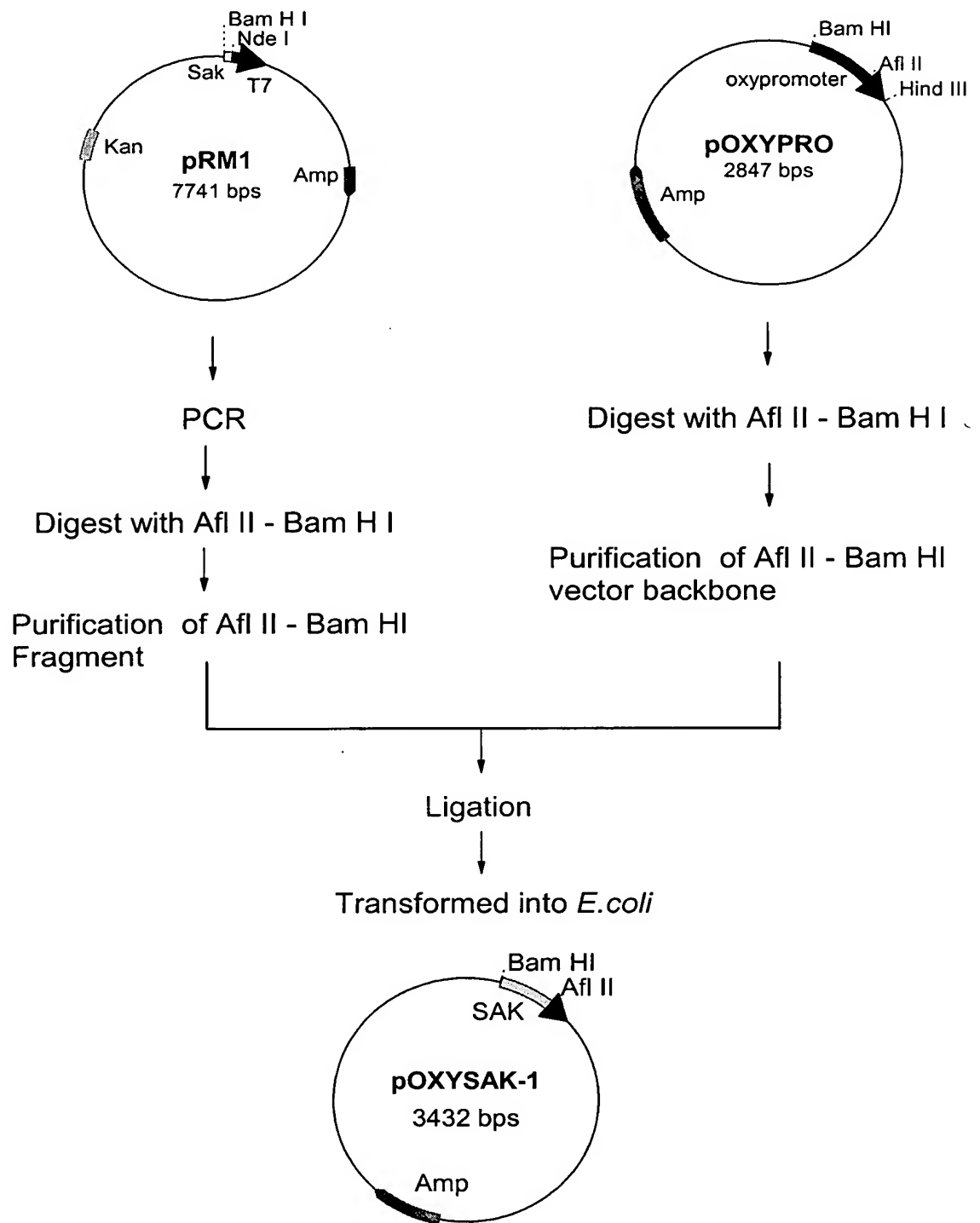


Fig.9 Construction and map of plasmid , pOXYSAK-2 containing SAK-2 gene under OXY-1 expression cassette.

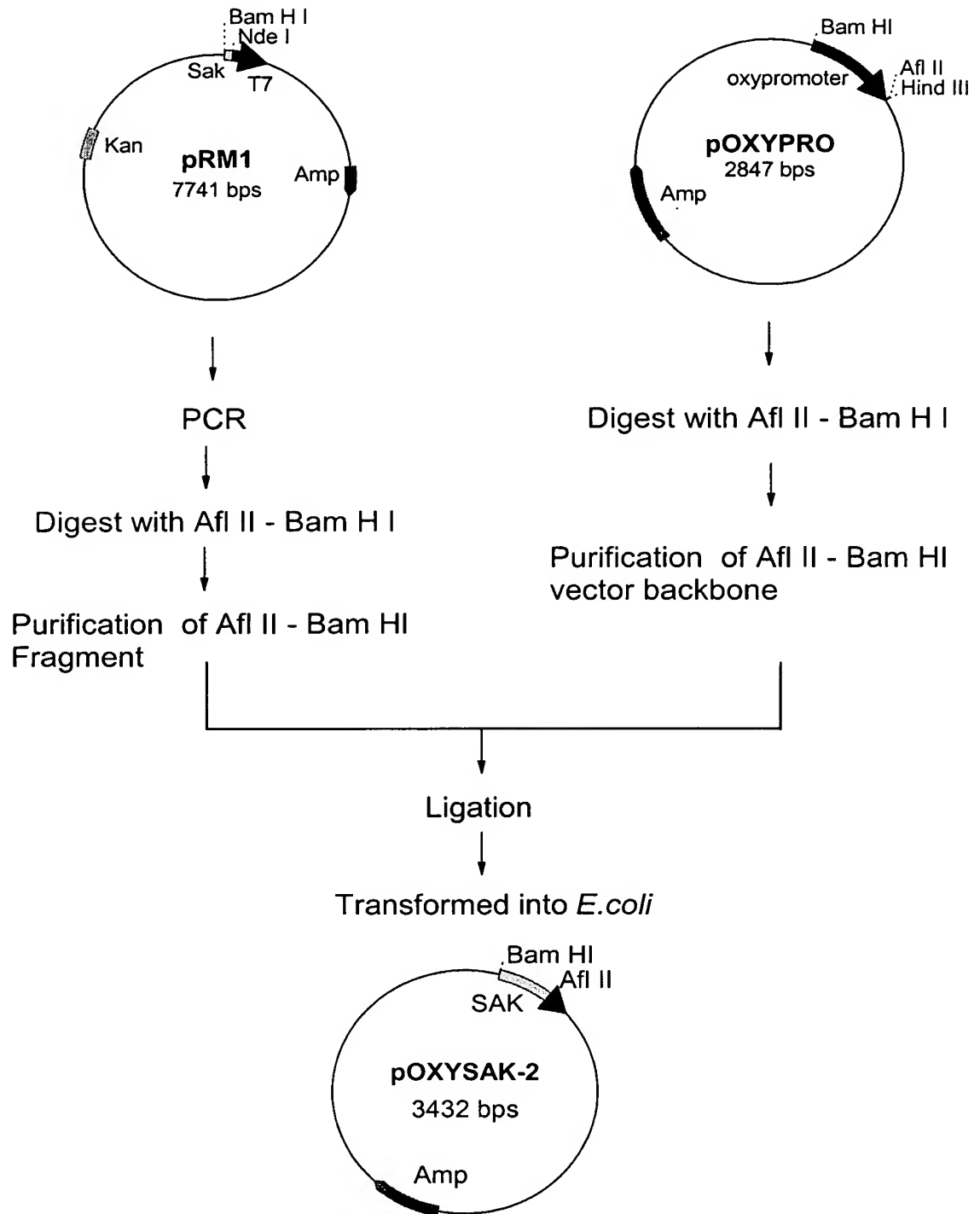


Fig.10 Sequences of SAK, SAK-1 and SAK-2 genes.

Terminator sequence in SAK-1 and SAK-2 are shown in bold letters and modified residues in SAK-2 are shown in small bold letters.

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5  SAK
   TCAAGTTCATTCGACAAAGGAAA
   SAK-1
   GAACTTAAGGAAGATATACATATGTCAAGTTCATTCGACAAAGGAAA
   SAK-2                               GAACTTAAGCATATG g c tGGA
10  gc

   SAK
   ATATAAAAAGGGCGATGACGCGAGTTATTTTGAACCAACAGGCCCGT
   SAK-1
15  ATATAAAAAGGGCGATGACGCGAGTTATTTTGAACCAACAGGCCCGT
   SAK-2                               t
   TATAAAAAGGGCGATGACGCGAGTTATTTTGAACCAACAGGCCCGT

   SAK
20  ATTTGATGGTAAATGTGACTGGAGTTGATGGTAAAGGAAATGAATTG
   SAK-1
   ATTTGATGGTAAATGTGACTGGAGTTGATGGTAAAGGAAATGAATTG
   SAK-2
   ATTTGATGGTAAATGTGACTGGAGTTGATGGTAAAGGAAATGAATTG
25

   SAK   CTATCCCCTCATTATGTCGAGTTTCCTATTAAACCTGGGACTACACT
   SAK-1 CTATCCCCTCATTATGTCGAGTTTCCTATTAAACCTGGGACTACACT
   SAK-2 CTATCCCCTCATTATGTCGAGTTTCCTATTAAACCTGGGACTACACT

30  SAK
   TACAAAAGAAAAAATTGAATACTATGTCTGAATGGGCATTAGATGCGA
   SAK-1
   TACAAAAGAAAAAATTGAATACTATGTCTGAATGGGCATTAGATGCGA

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SAK-2

TACAAAAGAAAAAATTGAATACTATGTCGAATGGGCATTAGATGCGA

SAK

5 CAGCATATAAAGAGTTTAGAGTAGTTGAATTAGATCCAAGCGCAAAG

SAK-1

CAGCATATAAAGAGTTTAGAGTAGTTGAATTAGATCCAAGCGCAAAG

SAK-2

CAGCATATAAAGAGTTTAGAGTAGTTGAATTAGATCCAAGCGCAAAG

10

SAK

ATCGAAGTCACTTATTATGATAAGAATAAGAAAAAGAAGAAACGAA

15 SAK-1

ATCGAAGTCACTTATTATGATAAGAATAAGAAAAAGAAGAAACGAA

SAK-2

ATCGAAGTCACTTATTATGATAAGAATAAGAAAAAGAAGAAACGAA

20

SAK

GTCTTTCCCTATAACAGAAAAAGGTTTTGTTGTCCCAGATTTATCAGA

SAK-1

25 GTCTTTCCCTATAACAGAAAAAGGTTTTGTTGTCCCAGATTTATCAGA

SAK-2

GTCTTTCCCTATAACAGAAAAAGGTTTTGTTGTCCCAGATTTATCAGA

SAK GCATATTAAAAACCCTGGATTCAACTTAATTACAAAGGTTGTTATAG

30 SAK-1

GCATATTAAAAACCCTGGATTCAACTTAATTACAAAGGTTGTTATAG

SAK-2

GCATATTAAAAACCCTGGATTCAACTTAATTACAAAGGTTGTTATAG

SAK AAAAGAAATAA

SAK-1

AAAAGAAATAAAACAAAATAGTTGTTTATTATAGAAAGTAATGTC

SAK-2

5 AAAAGAAATAAAACAAAATAGTTGTTTATTATAGAAAGTAATGTC

SAK-1

TTGATTGAATATGTGTAGTGAAATTATCTTTCATCAAATTCTCATT

SAK-2

10 TTGATTGAATATGTGTAGTGAAATTATCTTTCATCAAATTCTCATT

SAK-1

CATGCACGAATGGTTCTGCCCCACCTAATCAGATATTACGTGACT

SAK-2

15 CATGCACGAATGGTTCTGCCCCACCTAATCAGATATTACGTGACT

SAK-1

TATGGGGAGAAATCAGTTTGGATAAAAAGTGGAGGATCCAGTAGCC

20 SAK-2

TATGGGGAGAAATCAGTTTGGATAAAAAGTGGAGGATCCAGTAGCC

SAK-1 G

25 SAK-2 G

Fig. 11 Modification of SAK in SAK-2 . Modified residues are shown in bold letters.

	1	10	20	30	40
30	SAK				
	SSSFD K G K T K GDDASYFEPTGPYLMVNVTGVDG K GNE L LSPHYVEFP				
	SAK-2				
	AGAT K GDDASYFEPTGPYLMVNVTGVDG K GNE L LSPHYVEFP				

50 60 70 80 90

SAK

IKPGTTLTKEKIEYYVEWALDATAYKEFRVVELAPSAKIEVTYYDKNKK

SAK-2

5 IKPGTTLTKEKIEYYVEWALDATAYKEFRVVELAPSAKIEVTYYDKNKK

100 110 120 130 136

SAK EETTKSFPITEKGFVVPDLSEHIKNPGFNLITKVVIEKK

SAK-2 EETTKSFPITEKGFVVPDLSEHIKNPGFNLITKVVIEKK

10